View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by AJOL - African Journals Online

Alolga et al., Afr J Tradit Complement Altern Med., (2018) 15 (3): 18-26
https://doi.org/10.21010/ajtcam.v15i3.2

QUANTIFICATION OF XYLOPIC ACID AND CHROMATOGRAPHIC FINGERPRINT EVALUATIONS OF THE DRIED FRUITS OF *XYLOPIA AETHIOPICA* FROM FOUR AFRICAN COUNTRIES

Raphael N. Alolga[1*], Assogba G. Assanhou[2], Vitus Onoja[3]

[1]State Key Laboratory of Natural Medicines, Department of Pharmacognosy, China Pharmaceutical University, No. 639 Longmian Avenue, Nanjing 211198, China. [2]Université d'Abomey Calavi, Faculté des Sciences de la Santé, Unité de formation et de recherche en Pharmacie, département de pharmacie galénique et de biopharmacie, 07BP531. [3]Department of Pharmaceutics and Pharmaceutical Technology, University of Jos, Nigeria.

Corresponding Author's Email Address: anammahime@gmail.com; alolgaraph@yahoo.com; alolgara@cpu.edu.cn

Article History
Received: July, 28. 2017
Revised Received: Feb. 15, 2018
Accepted: Feb. 15. 2018
Published Online: May. 31, 2018

Abstract

Background: The fruits of *Xylopia aethiopica* (Dunal) A. Rich, (herein called XYA), family Annonaceae, commonly known as "Guinea pepper", "Ethiopian pepper" or "Negro pepper", are widely used in traditional African medicines to treat a wide array of diseases including malaria, fungal infections, rheumatism, arthritis, etc. Scientific investigations have ascribed the following activities to the fruits of XYA; anti-diabetic, anti-inflammatory, antimicrobial, antiplasmodial, analgesic, anti-nociceptive, anti-proliferative, spermatogenic and neuropharmacological effects. The main active principle reported is xylopic acid (XA), a kaurene diterpene. This study aimed to develop and validate a simple HPLC/UV (high performance liquid chromatography – ultraviolet detection) analytical method for the quantification of XA that can be reproduced in poor-resource settings where advanced analytical detection techniques such as HPLC-MS are unavailable.
Materials and Methods: Thus in this study, a simple C18 solid-phase extraction (SPE) column-pretreatment ─ HPLC/UV analytical procedure was developed for the quantification of XA in the dried fruits of XYA from four African countries, Benin, Cameroon, Ghana and Nigeria. The samples of XYA from the four countries were assessed for similarities using chromatographic fingerprinting.
Results: The HPLC method was validated for linearity, limits of detection and quantification, precision and accuracy. The samples of XYA from Cameroon were found to have the highest average content of XA while those from Benin had the lowest average content of XA.
Conclusion: Using the chromatographic fingerprint evaluation, the similarities of the samples from the four countries to the reference chromatogram was in the order: Benin > Cameroon > Nigeria > Ghana.

Key words: *Xylopia aethiopica*, xylopic acid, C18 solid-phase extraction (SPE) pre-treatment, HPLC/UV, chromatographic fingerprint evaluation

Abbreviations: C18: carbon 18, EMEA: European Medicines Agency, FDA: Food and Drugs Authority, HPLC-MS: high performance liquid chromatography- mass spectrometry, HPLC-NMR: high performance liquid chromatography- nuclear magnetic resonance, HPLC-UV: high performance liquid chromatography- ultraviolet, LOD: limit of detection, LOQ: limit of quantification, RSD: relative standard deviation, SPE: solid-phase extraction, S/N: signal to noise ratio, XA: xylopic acid XYA: Xylopia aethiopica

Introduction

The fruits of *Xylopia aethiopica* (Dunal) A. Rich, (herein referred to as XYA), family Annonaceae, commonly known as "Guinea pepper", "Ethiopian pepper" or "Negro pepper", are widely used in traditional African medicines and as spice. They are used to treat malaria, fungal infections, amenorrhoea, rheumatism, arthritis, hemorrhoids, diabetes, dysentery, bronchitis and flatulence. As a condiment in soup or tea, the fruits are also given to women after child birth to facilitate production of milk. The decoctions of the seeds are often used by traditional birth attendants to induce placental discharge postpartum.

Scientific investigations have ascribed the following activities to the fruits of XYA; anti-diabetic (Mohammed et al., 2016), anti-inflammatory (Obiri and Osafo, 2013, Obiri et al., 2014), antimicrobial (Coli, 2013, Ilusanya et al., 2012), antiplasmodial (Boyom et al., 2003), analgesic and anti-nociceptive (Ameyaw et al., 2014, Woode et al., 2016, Woode et al., 2012b), anti-proliferative (Adaramoye et al., 2017, Adaramoye et al., 2011, Choumessi et al., 2012), spermatogenic (Woode et al., 2011) and neuropharmacological (Biney et al., 2016, Biney et al., 2014) effects. The fruit extracts have also been found to offer protection against radiation in experimental animals (Adaramoye et al., 2010). Among these reported effects, xylopic acid (XA) has been found to be the main active principle responsible for the anti-nociceptive, anti-inflammatory and analgesic effects (Ameyaw et al., 2013, Woode et al., 2015) as well as antiplasmodial (Boampong et al., 2013) and spermatogenic (Woode et al., 2012a) effects. XA, i.e. 15β-acetoxy-(-)-kaur-16-en-19-oic acid, is a kaurene diterpene.

The XYA plant is mostly found in West, Central and Southern Africa. It is native to the lowland rainforest and moist fringe forest in the Savanna zones of Africa. It has been documented as a native plant in the following countries; Ghana, Ethiopia, Nigeria, Senegal, Democratic Republic of Congo, Tanzania, Uganda, Benin, Cameroon etc. Since differences in geographical location influences the quality of herbs or botanicals, it is worthwhile assessing the quality of herbs on a source-dependent manner with particular emphasis on the active compounds.

Chromatographic fingerprinting is a holistic identification method that reveals chemical information of botanical extracts with chromatograms and other graphical representations of analytical techniques. The use of chromatographic fingerprinting has gained popularity and acceptance by the WHO and both the U.S Food and Drugs Administration (FDA) and European Medicines Agency (EMEA) recommend its use in the quality consistency assessment of botanicals. The regulatory authority of China, the State Food and Drug Administration of China (SFDA) requires that all injections of plant origin be standardized by chromatographic fingerprinting. Additionally, the SFDA suggests that the chromatograms of botanicals be assessed for similarities usually based on the correlation coefficient of original data/chromatogram (Li et al., 2015). A combination of chromatographic fingerprinting and quantitative determination of the active compounds of botanicals provide a good degree of quality assurance.

Very few scientific reports have been made on the determination of the amount of XA in the fruits of XYA (Adosraku and Oppong Kyekyeku, 2011, Esuon, 2013). However, no report exists to the best of our knowledge on both the qualitative and quantitative assessment of different samples of the dried fruits of XYA from different countries. This study therefore aimed to compare the quality of the dried fruits of XYA from four countries in Africa, Benin, Cameroon, Ghana and Nigeria by determining the amount of XA in all batches using a simple C18 SPE column-pretreatment ─ HPLC method and determine their similarities using chromatographic fingerprinting. We also aimed primarily at developing a simple HPLC method that could be reproduced in poor-resource settings where access to advance detection techniques such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) in the case of HPLC-MS and HPLC-NMR remains a big challenge.

Materials and methods

Materials and chemicals

Six batches each of the dried fruits of XYA were purchased from the following markets in August, 2016: Missoke market, Douala, Littoral Province (Cameroon); Layinyan Koli main market, Gombe State (Nigeria); Adjegounle market, Cotonou (Benin) and Makola market, Accra (Ghana), identified by the first author (Dr. Raphael N. Alolga) based on macroscopic, organoleptic and phytochemical examinations and samples deposited at the herbarium of the Department of Pharmacognosy, China Pharmaceutical University. The following voucher numbers were assigned to the samples: 1001-1006/GHA/SKLN/CPU for the samples from Ghana; 1007-10012/CAM/SKLN/CPU for those from Cameroon; 10013-10018/BEN/SKLN/CPU for the samples from Benin and 10019-10024/NIG/SKLN/CPU for the samples from Nigeria. Xylopic acid (XA) (purity > 95%) was isolated from the dried fruits of XYA in our lab and its structure confirmed by spectroscopic methods. HPLC grade acetonitrile, methanol and formic acid were purchased from Sigma-Aldrich Co. (St. Louis, Missouri, USA). C18 solid-phase extraction columns (ENVI-18, 6 mL) were bought from Sigma-Aldrich Co. (St. Louis, Missouri, USA). Deionized water was prepared by Milli-Q system (Millipore, USA).

Apparatus and chromatographic condition

Shimadzu Prominence LC-20A HPLC system (Shimadzu Corporation, Kyoto, Japan) consisting a LC-20AT solvent delivery unit (quaternary pump), a DGU-20A5R degasser unit, a SIL-20A autosampler, a CTO-20A column oven and a SPD-20A UV-VIS detector was used. Chromatographic separation was achieved on an Agilent Poroshell 120 SB-C18 column (2.7 µm, 3.0 x 100 mm) with acetonitrile (A) and 0.1% (v/v) aqueous formic acid (B) as the mobile phases. An isocratic elution program of 70% acetonitrile (A) and 30% aqueous formic acid (0.1% v/v) was used and the absorbance monitored at 202 nm. The column temperature was maintained at 30 °C. An injection volume of 10 µL was used with five minutes post-run time after every three injections.

Sample and standard solution preparation

Each batch of the dried fruits of XYA was pulverized and sifted through a 250 µm sieve. 0.1g of the sieved powder was accurately weighed in a 10 mL Eppendorf tube into which 5 mL of methanol was added. The mixture was extracted via ultrasonication in an ultrasonic water bath (30 °C) at 100 Hz for 90 min. Following ultrasonication, the extracted solution was filtered through an analytical filter paper (Whatman No.1). For the similarity evaluation, the filtered samples underwent further filtration through a sintered glass filter of pore size 0.22 µm before injection into the HPLC system.

For the C18 SPE column pretreatment, the following protocol was used: the columns were first conditioned with 5 mL of methanol at a flow rate of 1 drop/sec and washed with 5 mL of water. 4 mL of each sample was then loaded onto the column. At also a flow rate of 1 drop/sec, the column was washed with 5 mL of water. The desired analyte was eluted with 3 mL of methanol, filtered through a 0.22 µm filter and dried under a gentle stream of nitrogen gas under room temperature. The dried sample was finally redissolved in 1 mL of methanol and a 10 µL aliquot injected into the HPLC system for analysis.

Stock solution of XA was prepared by dissolving an accurately weighed amount in methanol/DMSO (80: 20) to obtain 2 mg/mL. Standard solutions were prepared by serial dilutions of the stock solution stored at 4 °C before analysis.

Method validation

The developed method was validated for linearity, limits of detection and quantification, intra- and inter-day precision, and accuracy or recovery. The linear regression analysis of XA was obtained by plotting the peak areas (y) against the respective concentrations (x, mg/mL) of the standard solution. The limits of detection (LOD) and quantification (LOQ) were assessed by preparing further dilutions of the lowest concentration of the standard used for the linearity evaluation. The LOD was estimated as the minimum concentration of XA giving a signal of least 3 times stronger than the noise signal (i.e. signal to noise ratio, $S/N \geq 3$). The LOQ was determined as the minimum concentration that gave a signal of at least 10 times that of the noise signal ($S/N \geq 10$).

The precision of the method was assessed by investigating the intra-day and inter-day repeatability of three concentrations of the standard solution. For intra-day precision analysis (i.e. six injections each) of these samples were done at 4 h intervals within a single day while the analysis was done over a three-consecutive day period for inter-day precision. The recovery test was performed by analyzing spiked samples of three different concentrations of the standard solution.

Similarity evaluation

The similarities in the chromatograms of the various samples were analyzed with the software, Similarity Evaluation system for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A). Upon aligning all the peaks, the reference chromatogram was generated by reserving peaks above 0.1% of the percentage area. Peaks that existed in all chromatograms of the samples with reasonable heights and good resolutions were assigned as 'common peaks'. The total area of the common peaks must be more than 90% of the peak area of the whole chromatogram. Similarity evaluations were done for all samples from the four countries and among samples from the same countries to obtain respectively inter- and intra-countries' similarity values.

Results and discussion

With the aim of developing and validating a simple C18 SPE column-pretreatment ─ HPLC/UV method for the quantification of XA (structure shown in Fig. 1) in different samples of the dried fruits of XYA, as well as assess their similarities using chromatographic fingerprinting, the sample extraction procedure and chromatographic conditions were optimized.

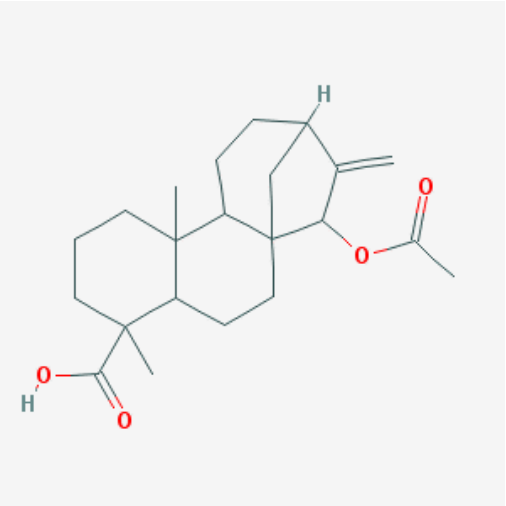

Figure 1: Chemical structure of xylopic acid (15β-acetoxy-(-)-kaur-16-en-19-oic acid)

Optimization of chromatographic conditions

Parameters such as solvent type, extraction method and time which usually influence extraction efficiency were optimized. The following solvents were tested, thus, methanol, water, ethyl acetate, dimethylsulfoxide (DMSO) and acetic acid at these time-points: 15, 30, 45, 60, 75, 90, 180, 240 min. Methanol was found to be the best solvent at 90 min of ultrasonication, giving high peak intensities with the lowest background noise. Ultrasonication was chosen due to its operational simplicity, availability and efficiency of extraction.

To obtain a better separation, the column, mobile phase composition and detection wavelength were investigated. Two columns were used for preliminary separations, Agilent Poroshell 120 SB-C18 column (2.7 µm, 3.0 x 100 mm) and Inertsil ODS-SP column (5 µm, 4.6 x 250 mm; GL Sciences Inc, Japan) and the former found to be better suitable giving good peak separations and stable baselines. The following mobile phase combinations were investigated; acetonitrile-water, acetonitrile ─ 0.1% (v/v) aqueous formic acid, methanol ─ water and methanol ─ 0.1% (v/v) aqueous formic acid. These solvent compositions were run in both isocratic and gradient elution systems and the best conditions being 70% methanol ─ 30% aqueous formic acid (0.1% v/v) in an isocratic elution with a short analytical time of 5 min. Due to the unique chemical structure of XA, the selection of an appropriate wavelength of absorption was quite challenging. The following wavelengths were tested, 254 nm, 342 nm, 202 nm and 220 nm. After a series of tests, 202 nm and 220 nm gave very good absorbances with little interference from the solvent but 202 nm was finally chosen due the consistency in absorbance over a wide concentration range.

These conditions are different from the previous reports. One study reported the following conditions: mobile phase, 90% methanol and 10% water in an isocratic elution at a flow rate of 0.5mL/min, injection volume of 20 µL and detection wavelength of 206 nm (Adosraku and Oppong Kyekyeku, 2011). Esuon employed similar conditions but the detection wavelength was 342nm (Esuon, 2013). However the retention time for XA from both studies was almost 5 min compared to 3.5 min for our study.

In order to clean up the samples, avoid a lot of interfering peaks and concentrate on the compound of interest, i.e., XA, the extracted samples underwent SPE column pretreatment prior to analysis. Using the chemical structure of XA, the logP value was calculated using the free online calculator (molinspiration.com/cgi-bin/properties). The logP value obtained for XA was approximately 5.44. A compound with a logP value > 4 is a good candidate for C18 SPE column extraction. Owing to its ready availability and low cost, the SPE column can be used in poor-resource settings. As can be seen from Fig.2, the chromatograms show the cleanup effect of the SPE column making the quantification of XA easier and much more reliable.

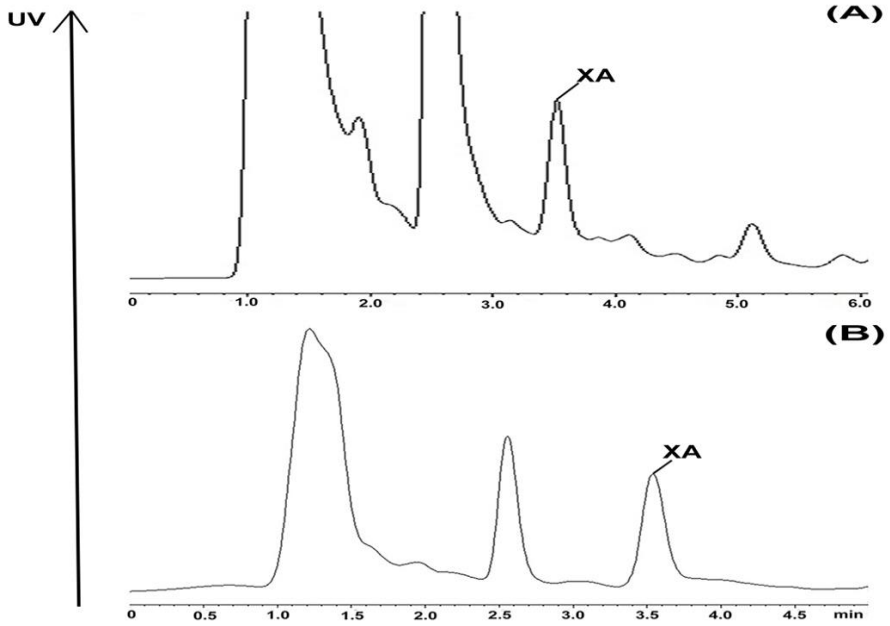

Figure 2: Chromatograms showing the clean-up effect of C18 solid-phase extraction (SPE) column. (A) Chromatogram of *Xylopia aethiopica* extract before SPE pretreatment. (B). Chromatogram of *Xylopia aethiopica* extract after SPE pretreatment.

Validation of method for quantitative analysis

The linearity of the method was observed in the concentration range of 0.2 ─ 0.00625 mg/mL with the correlation coefficient of 0.9993 from the calibration equation, y = 20000000x + 3849.7. The LOD and LOQ were respectively 0.024 µg/mL and 0.098 µg/mL. Satisfactory results of relative standard deviation (RSD) values in the range of 0.16% to 1.96% were obtained respectively for intra-day and inter-day precision. The results of recovery test were within the range of 98.20% and 102.50%. (Table 1).

Table 1: Outcome of validation of the analytical method

	Accuracy			Intra-day Precision	Inter-day Precision	
Conc. (mg/mL)	Added (µg)	Found (µg)	% Recovery	RSD (%, n= 6)	24 h RSD (%, n=6)	48 h RSD (%, n= 6)
0.05	100	98.2 ± 0.66	98.2	0.16 ± 0.24	1.12 ± 0.88	1.86 ± 0.96
0.1	100	99.1 ± 0.13	99.1	0.48 ± 0.18	0.68 ± 0.42	1.72 ± 1.02
0.15	100	102.5 ± 0.89	102.5	0.24 ± 0.41	1.02 ± 0.49	1.96 ± 0.99

Rt (min)	Calibration equation	Correlation coefficient	LOD (µg/mL)	LOQ (µg/mL)
3.503	y = 20000000x + 3849.7	0.9993	0.024	0.098

*Rt: Retention time of xylopic acid in minutes

Similarity evaluation of HPLC fingerprints

To evaluate the similarities between the various samples from the four countries by way of chromatographic fingerprint analysis, the software, *Similarity Evaluation system for Chromatographic Fingerprint of Traditional Chinese Medicine* (Version 2004A) was used. Similarity was reported in terms of cosine ratios. The closer the cosine ratio values are to unity, the more similar the samples are. The samples of dried XYA fruits from Benin showed the highest similarities to the reference chromatogram generated using all the samples from the four countries with values between 0.974 – 0.984. The samples from Cameroon also showed good similarities to the reference chromatogram with values between 0.963 – 0.971. The similarity values for the samples from Nigeria ranged from 0.816 to 0.969 while those from Ghana showed the least similarities to the reference chromatogram with values between 0.619 – 0.728. Thus in the order of similarities, the samples can be arranged as those from Benin > Cameroon > Nigeria > Ghana. (Figure 3, Table 2).

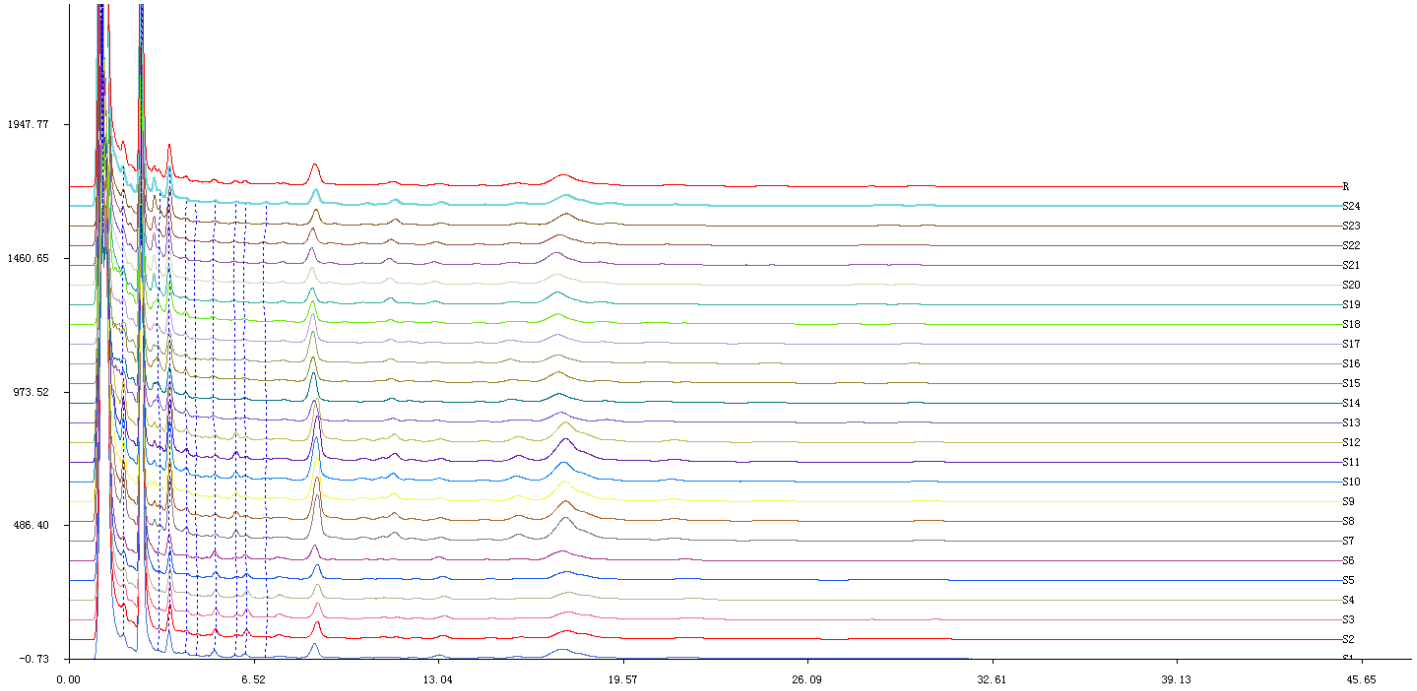

Figure 3: Chromatographic fingerprint of all samples from the four countries. S1-S6: Samples from Benin; S7-S12: Samples from Cameroon; S13-S18: Samples from Ghana; S19-S24: Samples from Nigeria.

Table 2: Results of inter-country similarity evaluations of *Xylopia aethiopica* samples

Batch	Country	Similarity
S1	Benin	0.983
S2	Benin	0.978
S3	Benin	0.974
S4	Benin	0.978
S5	Benin	0.982
S6	Benin	0.984
S7	Cameroon	0.971
S8	Cameroon	0.969
S9	Cameroon	0.965
S10	Cameroon	0.963
S11	Cameroon	0.971
S12	Cameroon	0.969

S13	Ghana	0.691
S14	Ghana	0.712
S15	Ghana	0.723
S16	Ghana	0.728
S17	Ghana	0.719
S18	Ghana	0.67
S19	Nigeria	0.969
S20	Nigeria	0.969
S21	Nigeria	0.864
S22	Nigeria	0.973
S23	Nigeria	0.816
S24	Nigeria	0.875

S1-S6: Samples from Benin; S7-S12: Samples from Cameroon; S13-S18: Samples from Ghana; S19-S24: Samples from Nigeria.

However the similarities among the samples from within the same countries were very high, almost near unity. The samples from Benin, Cameroon and Nigeria were similar in the range of 0.997 - 1 to the generated reference chromatograms while those from Ghana had similarity cosine ratio values of 0.996 – 0.999 (Supplementary Table S1). These results show that even though the various samples from the four countries differ on a country by country basis, they are very much similar from within each country.

Quantification of xylopic acid in samples of XYA

The content of XA (mg/g) in all samples of XYA from the four countries is summarized in Table 3. The samples from Cameroon had the highest average amount of XA with those from Benin having the lowest amount of XA. The order in terms of country with the highest content of XA to the lowest is thus; Cameroon > Ghana > Nigeria > Benin. The average amounts of XA (mg/g) from these countries are 0.7983, 0.5969, 0.5469 and 0.5302 for Cameroon, Ghana, Nigeria and Benin respectively. These results bring to the forefront the differential applications of the chromatographic fingerprint evaluations and the quantification of active constituents. The chromatographic fingerprint analysis gives a holistic evaluation of all constituents present in the given samples based on chromatograms obtained from the analytical method. It is therefore essential for qualitative purposes. The quantification of the active constituents provides additional details that cannot be capture using the chromatographic fingerprint alone. As can be seen from the results, the average amount of XA from the samples obtained from Ghana was even higher than those from Nigeria and Benin but showed the least similarities to the reference chromatogram. It is thus advisable to use the chromatographic fingerprint as a complementary method to the quantification of active compounds.

Table 3: Content of xylopic acid (XA) in all batches of *Xylopia aethiopica* from each country

Batch	Country	XA (mg/g)	Av. XA (mg/g)
SN1	Nigeria	1.0826 ± 0.12	
SN2	Nigeria	0.472 ± 0.02	
SN3	Nigeria	0.7201 ± 0.21	
SN4	Nigeria	0.1471 ± 0.18	
SN5	Nigeria	0.1973 ± 0.01	
SN6	Nigeria	0.6623 ± 0.06	0.5469
SC1	Cameroon	0.933 ± 0.04	
SC2	Cameroon	0.8535 ± 0.01	
SC3	Cameroon	0.7805 ± 0.09	
SC4	Cameroon	1.0181 ± 0.01	

SC5	Cameroon	0.9276 ± 0.05	
SC6	Cameroon	0.2773 ± 0.01	0.7983
SG1	Ghana	0.4787 ± 0.03	
SG2	Ghana	0.5433 ± 0.07	
SG3	Ghana	0.8339 ± 0.06	
SG4	Ghana	0.6117 ± 0.01	
SG5	Ghana	0.5488 ± 0.06	
SG6	Ghana	0.5655 ± 0.10	0.5969
SB1	Benin	0.8977 ± 0.01	
SB2	Benin	0.5357 ± 0.04	
SB3	Benin	0.4169 ± 0.05	
SB4	Benin	0.4922 ± 0.05	
SB5	Benin	0.7775 ± 0.03	
SB6	Benin	0.0611 ± 0.02	0.5302

*SN1-SN6: Samples from Nigeria; SC1-SC6: Samples from Cameroon; SG1-SG6: Samples from Ghana; SB1-SB6: Samples from Benin.

Conclusion

In summary, this study presents a simple C18 SPE column-pretreatment ─ HPLC/UV analytical method that can be readily reproduced in poor-resource settings where expensive advanced analytical detection techniques such as HPLC ─ MS and HPLC ─ NMR are unavailable. A total analytical time of 5 min was achieved. The method was validated for linearity, limits of detection and quantification, intra- and inter-day precision and accuracy. Using the chromatographic fingerprint evaluation, the similarities of the samples from the four countries to the reference chromatogram was in the order: Benin > Cameroon > Nigeria > Ghana. The samples from Cameroon were found to have the highest average content of XA while those from Benin had the lowest average content of XA.

Conflict of Interest: The authors declare no conflict of interest.

Acknowledgments: We wish to thank all who assisted us in obtaining the samples of *Xylopia aethiopica* (Dunal) A. Rich, particularly Nasiru Sintali, Dr. Irma Belinda and Majolene Sabi-Mouka.

References

1. Adaramoye, O. A., Erguen, B., Nitzsche, B., Hopfner, M., Jung, K., and Rabien, A. (2017). Antioxidant and antiproliferative potentials of methanol extract of Xylopia aethiopica (Dunal) A. Rich in PC-3 and LNCaP cells. Journal of Basic Clinical Physiology and Pharmacology., *28*: 403-412.
2. Adaramoye, O. A., Okiti, O. O., and Farombi, E. O. (2010). Dried fruit extract from Xylopia aethiopica (Annonaceae) protects Wistar albino rats from adverse effects of whole body radiation. Experimental and Toxicologic Pathology., *63:* 635-643.
3. Adaramoye, O. A., Sarkar, J., Singh, N., Meena, S., Changkija, B., Yadav, P. P., Kanojiya, S., and Sinha, S. (2011). Antiproliferative action of Xylopia aethiopica fruit extract on human cervical cancer cells. Phytotherapy Research., *25*: 1558-1563.
4. Adosraku, R., and Oppong Kyekyeku, J. (2011). Characterisation and HPLC quantification of xylopic acid in the dried fruits of xylopia aethiopica. International Journal of Pure and Applied Chemistry., *6*: 209-213.
5. Ameyaw, E., Boampong, J., Kukuia, K., Amoateng, P., Obese, E., Osei-Sarpong, C., and Woode, E. (2013). Effect of xylopic acid on paclitaxel-induced neuropathic pain in rats. Journal of Medical and Biomedical Sciences., *2*: 6-12.
6. Ameyaw, E. O., Woode, E., Boakye-Gyasi, E., Abotsi, W. K., Kyekyeku, J. O., and Adosraku, R. K. (2014). Anti-allodynic and Anti-hyperalgesic effects of an ethanolic extract and xylopic acid from the fruits of Xylopia aethiopica in murine models of neuropathic pain. Pharmacognosy Research., *6*: 172-179.
7. Biney, R. P., Benneh, C. K., Ameyaw, E. O., Boakye-Gyasi, E., and Woode, E. (2016). Xylopia aethiopica fruit extract exhibits antidepressant-like effect via interaction with serotonergic neurotransmission in mice. Journal of Ethnopharmacology., *184*: 49-57.

8. Biney, R. P., Mantel, P. K., Boakye-Gyasi, E., Kukuia, K. E., and Woode, E. (2014). Neuropharmacological effects of an ethanolic fruit extract of Xylopia aethiopica and xylopic acid, a kaurene diterpene isolate, in mice. West African Journal of Pharmacy., *25:* 106-117.
9. Boampong, J. N., Ameyaw, E. O., Aboagye, B., Asare, K., Kyei, S., Donfack, J. H., and Woode, E. (2013). The Curative and Prophylactic Effects of Xylopic Acid on Plasmodium berghei Infection in Mice. Journal of Parasitology Research., *2013*: 356107.
10. Boyom, F. F., Ngouana, V., Zollo, P. H., Menut, C., Bessiere, J. M., Gut, J., and Rosenthal, P. J. (2003). Composition and anti-plasmodial activities of essential oils from some Cameroonian medicinal plants. Phytochemistry., *64:* 1269-1275.
11. Choumessi, A. T., Danel, M., Chassaing, S., Truchet, I., Penlap, V. B., Pieme, A. C., Asonganyi, T., Ducommun, B., and Valette, A. (2012). Characterization of the antiproliferative activity of Xylopia aethiopica. Cell Division., *7*: 8-8.
12. Coli, A. U. O. E. (2013). Antimicrobial activity of Xylopia aethiopica (UDA) on Escherichia coli and Staphylococcus aureus isolates from gastroenteritic patients. International Journal of Life Sciences Biotechnology and Pharma Research.,2: 330-338.
13. Esuon, F. (2013). Extraction and Characterization of Xylopic Acid from Xylopia aethiopica [dissertation] . Johnson City, Tennessee: East Tennessee State University.
14. Ilusanya, O., Odunbaku, O., Adesetan, T., and Amosun, O. (2012). Antimicrobial activity of fruit extracts of Xylopia aethiopica and its combination with antibiotics against clinical bacterial pathogens. Journal of Biology, Agriculture and Healthcare., *2:* 1-9.
15. Li, J., He, X., Li, M., Zhao, W., Liu, L., and Kong, X. (2015). Chemical fingerprint and quantitative analysis for quality control of polyphenols extracted from pomegranate peel by HPLC. Food Chemistry., *176*: 7-11.
16. Mohammed, A., Koorbanally, N. A., and Islam, M. S. (2016). Anti-diabetic effect of Xylopia aethiopica (Dunal) A. Rich. (Annonaceae) fruit acetone fraction in a type 2 diabetes model of rats. Journal of Ethnopharmacology., *180*: 131-139.
17. Obiri, D. D., and Osafo, N. (2013). Aqueous ethanol extract of the fruit of Xylopia aethiopica (Annonaceae) exhibits anti-anaphylactic and anti-inflammatory actions in mice. Journal of Ethnopharmacology., *148*: 940-945.
18. Obiri, D. D., Osafo, N., Ayande, P. G., and Antwi, A. O. (2014). Xylopia aethiopica (Annonaceae) fruit extract suppresses Freund's adjuvant-induced arthritis in Sprague-Dawley rats. Journal of Ethnopharmacology., *152*: 522-531.
19. Woode, E., Alhassan, A., and Abaidoo, C. S. (2011). Effect of ethanolic fruit extract of Xylopia aethiopica on reproductive function of male rats. International Journal of Pharmaceutical and Biomedical Research., *2*: 161-165.
20. Woode, E., Alhassan, A., and Abaidoo, C. S. (2012a). Effect of xylopic acid on sex hormones and spermatogenesis in male rats. Al Ameen Journal of Medical Sciences., *5*: 288-297.
21. Woode, E., Ameyaw, E. O., Abotsi, W. K. M., and Boakye-Gyasi, E. (2015). An isobolographic analysis of the antinociceptive effect of xylopic acid in combination with morphine or diclofenac. Journal of Basic and Clinical Pharmacy., *6:* 103-108.
22. Woode, E., Ameyaw, E. O., Boakye-Gyasi, E., Abotsi, W. K., Oppong Kyekyeku, J., Adosraku, R., and Biney, R. P. (2016). Effects of an ethanol extract and the diterpene, xylopic acid, of Xylopia aethiopica fruits in murine models of musculoskeletal pain. Pharmaceutical Biology., *54*: 2978-2986.
23. Woode, E., Ameyaw, E. O., Boakye-Gyasi, E., and Abotsi, W. K. M. (2012b). Analgesic effects of an ethanol extract of the fruits of Xylopia aethiopica (Dunal) A. Rich (Annonaceae) and the major constituent, xylopic acid in murine models. Journal of Pharmacy and Bioallied Sciences., *4*: 291-301.